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# Dietary conjugated linoleic acid alters fatty acid composition of pig skeletal muscle and fat<sup>1,2,3</sup>

T. G. Ramsay<sup>\*4</sup>, C. M. Evock-Clover<sup>\*</sup>, N. C. Steele<sup>\*</sup>, and M. J. Azain<sup>†</sup>

<sup>\*</sup>USDA-ARS, Beltsville, MD 20705 and <sup>†</sup>University of Georgia, Athens 30602

**ABSTRACT:** The dietary dose responsiveness of conjugated linoleic acid (CLA) addition relative to the fatty acid profile of edible lean tissue was examined in grower pigs treated with or without porcine somatotropin (pST). Gilts and barrows were fed CLA at 0, 0.25, 0.5, 1.0, or 2.0% of diet by weight from 20 to 55 kg BW. Additional pigs were administered (pST) at 0 or 100  $\mu\text{g}\cdot\text{kg BW}\cdot\text{d}^{-1}$  and fed either 0.5 or 2.0% CLA. Animals were fed diets containing 18% CP, 1.2% lysine, and 3.5 Mcal of DE/kg at 110% of ad libitum intake. The fatty acid profile in latissimus dorsi and dorsal s.c. adipose tissue samples was determined by gas chromatography. Dietary CLA replacement of corn oil increased the percentage of total fatty acids as stearic acid, whereas the percentages as oleic and linolenic acids were reduced

in latissimus muscle. Treatment with CLA + pST increased the percentages of linoleic and arachidonic acids while reducing the percentages of palmitic and oleic acids in latissimus muscle. Dietary CLA increased the percentages of palmitic and stearic acids in s.c. adipose tissue while reducing the percentages of oleic, linoleic, linolenic, and arachidonic acids. The percentage of palmitic acid was reduced in s.c. adipose tissue, whereas linoleic acid was increased with CLA + pST. No synergistic effect was detected between CLA and pST for reducing carcass lipid content in grower pigs. However, pST increased the percentage of polyunsaturated fatty acids in latissimus muscle and s.c. adipose tissue while reducing the percentages of saturated fatty acids in swine fed CLA.

Key Words: Carcass Composition, Fatty Acids, Linoleic Acid

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## Introduction

Conjugated linoleic acid (CLA) is a collective term to describe positional and geometric isomers of linoleic acid (9,12-*cis*, *cis*-octadecadienoic acid) with conjugated double bonds at carbon 10 and 12 or 9 and 11, and all possible *cis* and *trans* combinations. Dietary CLA has been reported to reduce carcass fat (Dugan et al., 1997; Thiel et al., 1998) and to increase protein accretion rate (Ostrowska et al., 1999a) in finishing pigs.

Dietary CLA has also been associated with increased firmness of bellies from finishing pigs (Eggert et al.,

1999), suggesting an increased saturated fatty acid content. However, changes in fatty acid composition of the carcass have not been quantified and effects on grower pigs have not been reported.

Porcine somatotropin (pST) treatment can also reduce carcass fat accretion, increase lean deposition (Etherton and Smith, 1991), and alter fatty acid composition of porcine adipose tissue by increasing the percentage of unsaturated fatty acids (Clark et al., 1992; Kuhn et al., 1997). The present study was designed to determine whether the combination of pST and CLA could additively affect carcass fat content in grower pigs and to determine whether pST could counteract the effects of CLA on carcass saturated fat content.

## MATERIALS AND METHODS

### Animals

Forty-eight crossbred pigs (Yorkshire  $\times$  Landrace), half barrows and half gilts, were used to determine the effects of CLA in the diet, with or without pST administration, on a variety of measurements. These measurements included growth performance, serum hormone and metabolite concentrations, overall carcass component accretion, and alterations of fatty acid pro-

<sup>1</sup>Mention of a trade name, vendor, proprietary product or specific equipment is not a guarantee or a warranty by the U. S. Department of Agriculture and does not imply an approval to the exclusion of other products or vendors that also may be suitable.

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<sup>4</sup>Correspondence: Growth Biology Laboratory, BARC-East, Bldg. 200, Rm. 201 (phone: (301) 504-5958; fax: (301) 504-8623; E-mail: tramsay@psi.barc.usda.gov).

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files in muscle and adipose tissue samples. The pigs within a sex were randomly allotted to treatments at 20 kg BW and maintained on these treatments until 55 kg BW. The eight treatment groups ( $n = 7$  per group) were as follows: 1) controls, which received only the basal diet; 2) basal diet with 0.25% CLA; 3) basal diet with 0.5% CLA; 4) basal diet with 1.0% CLA; 5) basal diet with 2.0% CLA; 6) pST-controls, which received basal diet and were injected i.m. daily with sterile 50 mM bicarbonate buffer; 7) 0.5% CLA diet with daily i.m. injections of pST at a concentration of 100  $\mu\text{g/kg}$  BW; and 8) 2.0% CLA with daily i.m. injections of pST at a concentration of 100  $\mu\text{g/kg}$  BW. All procedures performed on these animals were approved by the Beltsville Area Swine Care and Use Committee. The CLA used in this study was produced by Bioriginal (Saskatoon, SK, Canada). The CLA contained 67% total CLA with approximately 25% of the *cis* 9,*trans* 11 isomer and 35% of the *cis* 10, *trans* 12 isomer (from certificate of analysis provided by the manufacturer). The relative fatty acid composition of the diets, including the two CLA isomers, is presented in Figure 1. Pigs were individually penned in environmentally controlled housing. Animals were individually fed a basal diet containing 18% CP, 1.2% lysine, and 3.5 Mcal of DE/kg (Table 1) at 110% of ad libitum intake (ARC, 1981), with access to feed from 0800 to 1300. Feed intake was measured and recorded daily. We have found that the ARC equation underestimates ad libitum intake for American swine breeds, and thus a 10% increase in quantity of

**Table 1.** Composition of the basal diet (as-fed basis)<sup>a</sup>

Ingredient	Percentage of total
Corn	61.8
Soybean meal (48% CP)	18.0
Dried skim milk	12.0
Calcium carbonate	2.5
Sodium phosphate, monobasic	2.5
Corn oil <sup>b</sup>	2.0
Iodized salt	0.5
Lysine hydrochloride	0.25
Mineral premix, swine complete mix <sup>c</sup>	0.2
Swine vitamin mix <sup>c</sup>	0.2
Selenium premix <sup>d</sup>	0.05

<sup>a</sup>Calculated nutrient composition: 18% CP; 1.2% lysine; 3.5 Mcal of DE/kg.

<sup>b</sup>The various percentages of CLA were added in place of that amount of corn oil.

<sup>c</sup>For actual composition of mineral and vitamin premixes, see Campbell et al. (1988).

<sup>d</sup>Provided 22  $\mu\text{g}$  of selenium/kg diet.

feed was provided. The basal and CLA-containing diets were mixed at and donated by United Feeds (Sheridan, IN). Pigs were weighed weekly, and pST and feed intake were adjusted weekly on an individual pig BW basis.

### Somatotropin

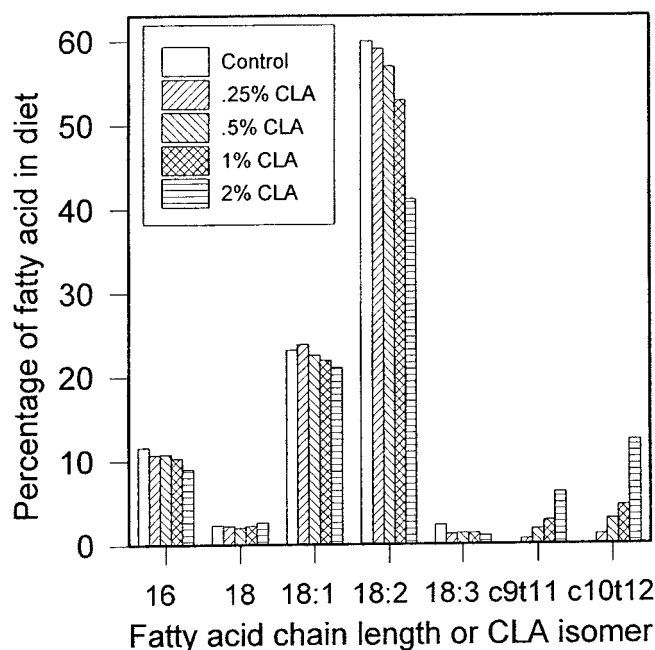
The pST was purchased from Southern Cross Biotech (Toorak, Victoria, Australia) and was supplied as a powder of 50% pST and 50% bicarbonate buffer. The pST was reconstituted in sterile, nonpyrogenic water 1 d before use. All pigs were injected with a dosage of 100  $\mu\text{g/kg}$  BW in the extensor neck muscle between 0800 and 0830 before feeding. This dosage was based on previous studies in pigs of this size (Campbell et al., 1988; Caperna et al., 1990; Steele et al., 1995).

### Blood Collection and Analysis

Blood samples were obtained from each pig by venipuncture in the vena cava just before pigs were fed at 0800 or injected with pST, and again at 1100 on the day before slaughter at 55 kg BW. Serum was collected and analyzed for several hormones and metabolites. Insulin was measured via RIA (Steele et al., 1985). Iodinated porcine insulin was obtained from Bynax (Portland, ME). Insulin-like growth factor I (IGF-I) was measured by RIA (Ballard et al., 1990), except that 20  $\mu\text{L}$  of pig serum was extracted with 80  $\mu\text{L}$  of PBS and 400  $\mu\text{L}$  of 0.2 N HCl: absolute ethanol (1:8). This mixture was then neutralized with 200  $\mu\text{L}$  of 0.855 M Tris base. Recombinant human IGF-I (R & D Systems, Minneapolis, MN) was used as the standard. Serum glucose and urea nitrogen (Sigma Chemical, St. Louis, MO) and nonesterified fatty acids (NEFA; Wako Diagnostics, Richmond, VA) were determined with kit assays.

### Carcass Components

Pigs were stunned by electric shock (400 V for 30 to 60 s) and then killed by exsanguination. Organ weights



**Figure 1.** Percentage of individual fatty acids and conjugated linoleic acid (CLA) isomers (c9:t11, c10:t12) constituting the total measurable fatty acid pool in samples of the diets containing 0 to 2.0% CLA. Myristic, palmitoleic, and arachidonic acids were undetectable in the feed.

were obtained for the heart, liver, and kidney. The right side of each carcass was chilled overnight, and standard carcass measurements were recorded. The half-carcass was then ground in entirety and samples were frozen for carcass analysis. Standard AOAC (1970) methods were used to determine carcass water, nitrogen, and ash contents. Carcass lipid was extracted and measured gravimetrically (Folch et al., 1957).

### Fatty Acid Profiles

The fatty acid profiles of the diet, lattissimus dorsi, and dorsal s.c. adipose tissue samples were determined by gas chromatography using a Shimadzu gas chromatograph (Model 14 A, Tokyo, Japan) with a flame ionization detector. Tissue samples were collected from the 10th rib on the left side of the carcass. A complete cross-section of s.c. adipose was harvested, diced, and frozen in liquid nitrogen. A complete cross-section of longissimus dorsi directly underneath the adipose sample was also collected, diced, and frozen in liquid nitrogen.

Samples of the diced adipose tissue (~100 mg), diced muscle (~2 g), or diet (1 g) were saponified and methylated in duplicate using procedures described previously (Azain, 1993; Azain et al., 2000). Heptadecanoic acid was used as an internal standard. Fatty acid methyl esters in hexane were separated on a Supelcowax-10 fused capillary column (60 m × 0.53 mm, 0.5-mm film thickness; Supelco, Bellefonte, PA) under isothermal conditions. Column temperature was 240°C, injector temperature was 250°C, and detector temperature was 260°C. Injection volume was 0.5 µL and helium was the carrier gas. Peak identification was based on known standards that included pure samples of *cis* 9, *trans* 11 and *trans* 10, *cis* 12 CLA (Matreya, Pleasant Gap, PA). Under these conditions, the *cis* 9, *trans* 11 (and *trans* 9, *cis* 11) isomer elutes after α-linolenic acid (18:3 *n*-3) and is followed by the *trans* 10, *cis* 12 isomer (Ha et al., 1989). For newer and improved methods of CLA analysis, please refer to Yurawecz et al. (1999).

### Statistics

Statistical analyses were performed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). The initial model included sex, treatment, and any interactions. Since sex was only significant for the gain:feed ratio and blood urea N and IGF-I, all other data were reanalyzed without sex in the model. Two different models were then used. The first model only included the five CLA treatments alone. The next model included the three pST treatments with their corresponding CLA treatments, for a total of six treatments, and these were then analyzed with pST, CLA, and the interactions in the model. The experimental unit was the pig. The Bonferroni method was used to compare treatment means. Also, for the serum data, time and any additional interactions were included in the above-mentioned models.

## Results

Neither dietary CLA nor pST affected total gain or the number of days on the experiment ( $P \geq 0.05$ ). Dietary CLA had no effect on ADG or feed efficiency ( $P \geq 0.05$ , Table 2). Animals treated with pST had reduced feed intakes ( $P < 0.01$ ) relative to their comparative CLA-only treatment groups, resulting in improved feed efficiency ( $P < 0.005$ ). Gilts had lower feed efficiency than barrows ( $P < 0.01$ ).

Serum glucose concentrations were unchanged ( $P \geq 0.05$ ) by CLA treatment or by sex of the animal at either time measured (Table 3). Glucose levels in pigs administered pST were elevated ( $P < 0.05$ ) during both the unfed and fed time points, relative to control animals or those receiving the same percentage of CLA in their diet but no pST injection. The one exception to this was the unfed 2% CLA + pST treatment group, which did not exhibit an increase in serum glucose concentration compared to the group receiving 2% CLA alone. Overall, feeding increased serum glucose concentrations over those obtained from unfed animals ( $P < 0.001$ ). Serum insulin was not affected by dietary CLA supplementation (Table 3). Animals in the fed state had much higher serum insulin levels than those in the unfed state ( $P < 0.001$ ). Treatment with pST dramatically elevated serum insulin following feeding ( $P < 0.001$ ). Blood urea nitrogen (BUN) was higher in the fed state for all treatment groups (Table 3). Dietary CLA had no effect on BUN ( $P \geq 0.05$ ). Barrows had higher BUN values than gilts ( $P < 0.002$ ). Treatment with pST reduced BUN values in swine ( $P < 0.002$ ). Serum NEFA were unaffected by CLA treatment, feeding state, or pST treatment (data not presented). Dietary CLA did not affect serum IGF-I levels in unfed animals ( $P \geq 0.05$ , Table 3). Serum IGF-I concentrations were higher in barrows than in gilts ( $P < 0.023$ ). Somatotropin treatment elevated serum IGF-I levels ( $P < 0.001$ ).

Hot carcass weight, carcass length and dressing percentage were unaffected ( $P \geq 0.05$ ) by CLA addition to the diet or by pST administration (Table 4). Low levels of dietary CLA (0.25 and 0.5%) increased ( $P < 0.05$ ) 10th rib backfat depth in comparison to the control group, although there was no effect detected in comparison to the buffer-injected animals. Treatment with pST reduced 10th rib backfat thickness ( $P < 0.05$ ). This was reflected in a reduction in percentage of carcass lipid in pST-treated animals ( $P < 0.05$ , Table 5). Water percentage was increased in the carcasses of animals treated with pST ( $P < 0.05$ ). No effects ( $P \geq 0.05$ ) of pST on carcass percentage of protein or ash were observed. Dietary CLA had no effect ( $P \geq 0.05$ ) on liver, heart, or kidney weights expressed as a percentage of body weight (Table 6). Somatotropin treatment increased liver and kidney weights ( $P < 0.05$ ). Barrows had larger livers than gilts ( $P < 0.05$ ).

Dietary CLA produced changes ( $P < 0.05$ ) in lattissimus muscle and dorsal s.c. adipose tissue fatty acid composition. The CLA content of muscle tissue in-



**Table 2.** Effects of dietary conjugated linoleic acid and(or) porcine somatotropin treatment on the growth performance of grower pigs

Item	CNT	Treatment group <sup>a</sup>							Pooled SEM	AOV <sup>b</sup>
		0.25%C	0.5%C	1%C	2%C	BUF	0.5%+P	2%+P		
Initial wt, kg	18.25	18.25	18.83	18.58	18.58	18.58	18.67	18.08	0.74	—
Final wt, kg	54.50	54.92	54.42	53.92	54.33	56.33	55.58	54.67	0.57	—
Days on trial	48.5	47.8	48.0	47.0	49.3	48.7	47.2	48.5	2.4	—
ADG, g	760	776	742	754	735	785	788	799	29	—
Feed intake, kg	76.7 <sup>w</sup>	78.0 <sup>w</sup>	76.9 <sup>w</sup>	76.0 <sup>w</sup>	76.8 <sup>w</sup>	77.0 <sup>w</sup>	69.8 <sup>x</sup>	69.0 <sup>x</sup>	1.8	P
Gain:feed	0.472 <sup>wx</sup>	0.469 <sup>wx</sup>	0.463 <sup>w</sup>	0.454 <sup>w</sup>	0.454 <sup>w</sup>	0.495 <sup>wx</sup>	0.529 <sup>x</sup>	0.529 <sup>x</sup>	0.013	Tr, P
Barrow	0.493	0.510	0.472	0.450	0.476	0.488	0.538	0.532	—	Sex
Gilt	0.454	0.435	0.452	0.459	0.433	0.500	0.521	0.526	—	—

<sup>a</sup>Treatment groups: CNT, fed control diet; 0.25%C, 0.25% CLA in diet; 0.5%C, 0.5% CLA in diet; 1%C, 1% CLA in diet; 2%C, 2% CLA in diet; BUF, fed control diet and i.m. injected daily with vehicle; 0.5%C+P, 0.5% CLA in diet and i.m. injected daily with 100 µg of recombinant porcine somatotropin/kg BW; 2%C+P, 2% CLA in diet and i.m. injected daily with 100 µg of recombinant porcine somatotropin/kg BW.

<sup>b</sup>Tr = treatment effect, Sex = sex effect (when run as eight treatments), and P = somatotropin effect.

<sup>w,x</sup>Within a row, means lacking a common superscript letter differ ( $P < 0.05$ ).

creased ( $P < 0.05$ ) with increasing dietary CLA content (Figure 2). The 10,12 isomer was in greater concentration than the 9,11 isomer ( $P < 0.05$ ), similar to the differences between the diets (Figure 1). Dietary CLA at 1 or 2% increased ( $P < 0.05$ ) the percentage of total fatty acids as stearic acid (Figure 3), whereas 2% CLA reduced ( $P < 0.05$ ) the percentage as oleic acid in muscle, reflecting the reduction in dietary oleic acid with increasing CLA content. Muscle linolenic acid seemed to be most sensitive to replacement of dietary corn oil with CLA, because linolenic acid was reduced in the

latissimus dorsi ( $P < 0.05$ ) with as little as 0.25% dietary CLA. Treatment with pST increased ( $P < 0.05$ ) the percentage of linoleic and linolenic acids that comprised the muscle fatty acids (Figure 4). Somatotropin treatment reduced ( $P < 0.05$ ) the percentages of palmitic and oleic acids in skeletal muscle.

The composition of fatty acids within s.c. adipose tissue following CLA treatment (Figures 5 and 6) and pST administration (Figure 7) differed from that in muscle. The CLA content of adipose tissue increased ( $P < 0.05$ ) with increasing dietary CLA content (Figure 5). The

**Table 3.** Effects of dietary conjugated linoleic acid and(or) porcine somatotropin treatment on serum hormones and metabolites of grower pigs

Item	CNT	Treatment group <sup>a</sup>							Pooled SEM	AOV <sup>b</sup>
		0.25%C	0.5%C	1%C	2%C	BUF	0.5%+P	2%+P		
Glucose, mg/dL										
0800	99 <sup>i</sup>	109 <sup>uvwx</sup>	106 <sup>uvw</sup>	102 <sup>i</sup>	102 <sup>i</sup>	99 <sup>i</sup>	122 <sup>xy</sup>	109 <sup>uvwx</sup>	5	Tr, P
1100	117 <sup>wx</sup>	121 <sup>xy</sup>	116 <sup>vwx</sup>	102 <sup>iv</sup>	106 <sup>uvw</sup>	104 <sup>uvw</sup>	138 <sup>z</sup>	136 <sup>yz</sup>		T
Insulin, pg/mL										
0800	71 <sup>u</sup>	65 <sup>u</sup>	110 <sup>uv</sup>	120 <sup>uv</sup>	72 <sup>u</sup>	111 <sup>uv</sup>	181 <sup>uv</sup>	112 <sup>uv</sup>	208	Tr, P
1100	520 <sup>uvw</sup>	552 <sup>uvw</sup>	842 <sup>v</sup>	688 <sup>vw</sup>	526 <sup>uvw</sup>	385 <sup>uvw</sup>	2,623 <sup>x</sup>	2,732 <sup>x</sup>		T, Tr × T, P × T
Urea nitrogen, mg/dL										
0800	7.9 <sup>uv</sup>	8.8 <sup>vwx</sup>	7.5 <sup>uv</sup>	7.0 <sup>uv</sup>	6.3 <sup>uv</sup>	8.4 <sup>vw</sup>	5.1 <sup>u</sup>	5.8 <sup>uv</sup>	1.0	Tr, P
Barrow	8.4	8.7	9.1	7.2	7.2	8.8	4.6	6.2		Sex
Gilt	7.4	8.9	6.0	6.7	5.3	8.0	5.7	5.5		
1100	13.0 <sup>y</sup>	13.1 <sup>y</sup>	13.4 <sup>y</sup>	13.2 <sup>y</sup>	10.9 <sup>wxy</sup>	11.4 <sup>xy</sup>	8.4 <sup>vw</sup>	8.5 <sup>vw</sup>		T
Barrow	13.0	12.4	17.2	15.0	12.2	12.9	8.9	9.9		
Gilt	13.0	13.9	9.6	11.5	9.7	9.9	7.9	7.2		
Insulin-like growth factor I, ng/mL										
0800	221 <sup>uvw</sup>	213 <sup>uvw</sup>	262 <sup>w</sup>	194 <sup>uv</sup>	212 <sup>uvw</sup>	264 <sup>w</sup>	451 <sup>x</sup>	412 <sup>x</sup>	22	Tr, P
Barrow	243	217	276	199	221	249	479	457		Sex
Gilt	198	209	249	189	204	280	424	368		Tr × Sex

<sup>a</sup>Treatment groups: CNT, fed control diet; 0.25%C, 0.25% CLA in diet; 0.5%C, 0.5% CLA in diet; 1%C, 1% CLA in diet; 2%C, 2% CLA in diet; BUF, fed control diet and i.m. injected daily with vehicle; 0.5%C+P, 0.5% CLA in diet and i.m. injected daily with 100 µg of recombinant porcine somatotropin/kg BW; 2%C+P, 2% CLA in diet and i.m. injected daily with 100 µg of recombinant porcine somatotropin/kg BW.

<sup>b</sup>Tr = treatment effect, Sex = sex effect (when run as eight treatments), P = somatotropin effect, and T = time effect.

<sup>u,v,w,x,y,z</sup>Within a row, means lacking a common superscript letter differ ( $P < 0.05$ ). When a T effect is present, means between the 0800 and 1100 treatment groups lacking a common superscript letter also differ ( $P < 0.05$ ).

**Table 4.** Effects of dietary conjugated linoleic acid and(or) porcine somatotropin treatment on carcass parameters of grower pigs

Item	CNT	Treatment group <sup>a</sup>							Pooled SEM	AOV <sup>b</sup>
		0.25%C	0.5%C	1%C	2%C	BUF	0.5%+P	2%+P		
Carcass weight, kg	39.2	39.2	39.5	38.7	38.5	40.1	39.6	38.0	0.7	—
Carcass length, mm	672	666	663	661	664	671	683	669	8.0	—
Dressing %	72.0	71.4	72.5	71.8	70.8	71.2	71.3	69.5	1.2	—
10th Rib backfat, mm	7.8 <sup>wx</sup>	11.3 <sup>z</sup>	10.7 <sup>yz</sup>	8.2 <sup>wxy</sup>	9.0 <sup>xyz</sup>	8.8 <sup>xyz</sup>	5.5 <sup>v</sup>	5.8 <sup>vw</sup>	0.9	Tr, P
Loineye area, cm <sup>2</sup>	22.0	20.8	20.3	20.4	21.6	21.6	19.0	20.6	1.0	—

<sup>a</sup>Treatment groups: CNT, fed control diets; 0.25%C, 0.25% CLA in diet; 0.5%C, 0.5% CLA in diet; 1%C, 1% CLA in diet; 2%C, 2% CLA in diet; BUF, fed control diet and i.m. injected daily with vehicle; 0.5%C+P, 0.5% CLA in diet and i.m. injected daily with 100 µg of recombinant porcine somatotropin/kg BW; 2%C+P, 2% CLA in diet and i.m. injected daily with 100 µg of recombinant porcine somatotropin/kg BW.

<sup>b</sup>Tr = treatment effect and P = somatotropin effect.

<sup>v,w,x,y,z</sup>Within a row, means lacking a common superscript letter differ ( $P < 0.05$ ).

10,12 isomer was in greater concentration than the 9,11 isomer ( $P < 0.05$ ), similar to the differences between diets (Figure 1). Dietary CLA increased ( $P < 0.05$ ) the percentage of myristic, palmitic, and stearic acids as a percentage of the total fatty acids in s.c. adipose tissue (Figure 6). Replacement of dietary corn oil with CLA reduced ( $P < 0.05$ ) the percentage of oleic, linoleic, linolenic, and arachidonic acids at dietary concentrations of CLA as low as 0.25% of the diet, reflecting the change in diet fatty acid composition. Palmitic, oleic, and linolenic acids were the most sensitive to dietary CLA substitution of corn oil. The percentage of total fatty acids as palmitic acid was reduced ( $P < 0.05$ ) in adipose tissue, whereas linoleic acid was increased ( $P < 0.05$ ) with pST administration (Figure 7).

## Discussion

Dietary CLA supplementation has previously been reported to increase average daily gain (Thiel et al., 1998) and enhance feed efficiency in finishing swine (Thiel et al., 1998; Ostrowska et al., 1999a). However, this was not observed in some other studies (Dugan et al., 1997; Dunshea et al., 1998) or in the present experiment. This may have been the consequence of the rapid, efficient growth rate of the grower pigs in

the present experiment vs finisher pigs in the study of Thiel et al. (1998) or Ostrowska et al. (1999a). Alternatively, composition of the CLA mix in the diet or total fat content may have differed sufficiently to cause the variable response among these studies. Last, we cannot exclude the small sample size, although variability was not a consistent problem in this study. Treatment with pST reduced total feed intake and increased the gain:feed ratio in these grower pigs, as reported previously (Campbell et al., 1988; Evock-Clover, 1997), although it did not affect ADG or average days on the experiment relative to buffer-treated animals.

Analysis of serum hormones and metabolites revealed no apparent effects of CLA on measurements in pigs at 55 kg. Cook et al. (1998) also reported no effect on serum metabolites in pigs fed 0.48 or 0.95% CLA-60 for 98 d. Dietary CLA was shown to alter serum IGF-I and IGF binding protein concentrations in rats fed 1% CLA for 42 d (Li et al., 1999). However, the present study found no evidence that dietary CLA alters serum IGF-I in grower pigs. Dietary CLA fed at 1% of the diet was reported to produce an increase in serum insulin in unfed rodents (Delany et al., 1999). Insulin levels before feeding were elevated, although not statistically significant, in pigs provided diets containing 0.5

**Table 5.** Effects of dietary conjugated linoleic acid and(or) porcine somatotropin treatment on carcass chemical composition of grower pigs

Item	CNT	Treatment group <sup>a</sup>							Pooled SEM	AOV <sup>b</sup>
		0.25%C	0.5%C	1%C	2%C	BUF	0.5%+P	2%+P		
Water, %	60.0 <sup>w</sup>	59.1 <sup>w</sup>	59.0 <sup>w</sup>	59.5 <sup>w</sup>	60.0 <sup>w</sup>	59.6 <sup>w</sup>	64.1 <sup>x</sup>	64.0 <sup>x</sup>	0.6	Tr, P
DM, %	40.0 <sup>w</sup>	40.9 <sup>w</sup>	41.0 <sup>w</sup>	40.5 <sup>w</sup>	40.0 <sup>w</sup>	40.4 <sup>w</sup>	35.9 <sup>x</sup>	36.1 <sup>x</sup>	0.6	Tr, P
Protein, %	17.1	16.7	17.2	17.7	17.6	17.1	17.5	17.8	0.4	—
Lipid, %	18.4 <sup>w</sup>	19.8 <sup>w</sup>	18.6 <sup>w</sup>	18.8 <sup>w</sup>	17.8 <sup>w</sup>	18.5 <sup>w</sup>	13.5 <sup>x</sup>	13.5 <sup>x</sup>	0.7	Tr, P
Ash, %	3.0	3.3	3.3	3.2	3.1	3.1	3.2	3.5	0.2	—

<sup>a</sup>Treatment groups: CNT, fed control diet; 0.25%C, 0.25% CLA in diet; 0.5%C, 0.5% CLA in diet; 1%C, 1% CLA in diet; 2%C, 2% CLA in diet; BUF, fed control diet and i.m. injected daily with vehicle; 0.5%C+P, 0.5% CLA in diet and i.m. injected daily with 100 µg of recombinant porcine somatotropin/kg BW; 2%C+P, 2% CLA in diet and i.m. injected daily with 100 µg of recombinant porcine somatotropin/kg BW.

<sup>b</sup>Tr = treatment effect and P = somatotropin effect.

<sup>w,x</sup>Within a row, means lacking a common superscript letter differ ( $P < 0.05$ ).

**Table 6.** Effects of dietary conjugated linoleic acid and(or) porcine somatotropin treatment on selected organ weights (as a percentage of BW) of grower pigs

Item	CNT	Treatment group <sup>a</sup>							Pooled SEM	AOV <sup>b</sup>
		0.25%C	0.5%C	1%C	2%C	BUF	0.5%+P	2%+P		
Heart, %	0.345	0.344	0.351	0.364	0.383	0.381	0.379	0.401	0.022	—
Kidney, %	0.467 <sup>w</sup>	0.498 <sup>wx</sup>	0.465 <sup>w</sup>	0.471 <sup>w</sup>	0.484 <sup>w</sup>	0.499 <sup>wx</sup>	0.578 <sup>xy</sup>	0.598 <sup>y</sup>	0.017	Tr, P
Liver, %	1.63 <sup>w</sup>	1.63 <sup>w</sup>	1.60 <sup>w</sup>	1.57 <sup>w</sup>	1.70 <sup>w</sup>	1.70 <sup>w</sup>	1.80 <sup>wx</sup>	2.00 <sup>x</sup>	0.09	Tr, P, C
Barrow	1.82	1.80	1.63	1.61	1.81	1.82	1.77	2.09		Sex
Gilt	1.43	1.46	1.58	1.53	1.58	1.57	1.83	1.92	—	—

<sup>a</sup>Treatment groups: CNT, fed control diet; 0.25%C, 0.25% CLA in diet; 0.5%C, 0.5% CLA in diet; 1%C, 1% CLA in diet; 2%C, 2% CLA in diet; BUF, fed control diet and i.m. injected daily with vehicle; 0.5%C+P, 0.5% CLA in diet and i.m. injected daily with 100 µg of recombinant porcine somatotropin/kg BW; 2%C+P, 2% CLA in diet and i.m. injected daily with 100 µg of recombinant porcine somatotropin/kg BW.

<sup>b</sup>Tr = treatment effect, Sex = sex effect (when run as eight treatments), P = somatotropin effect, and C = CLA effect (both when run only with like treatment groups).

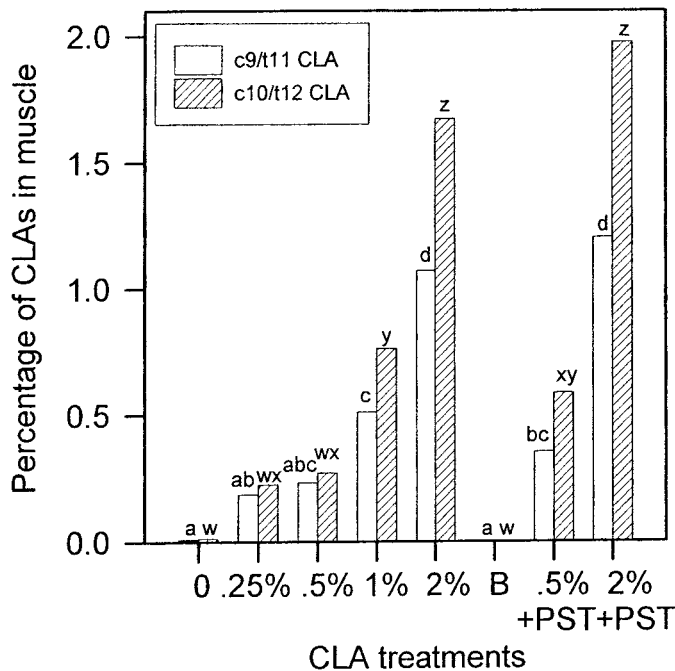
<sup>w,x,y</sup>Within a row, means lacking a common superscript letter differ ( $P < 0.05$ ).

or 1.0% CLA. These insulin levels remained elevated after feeding, although again not significantly.

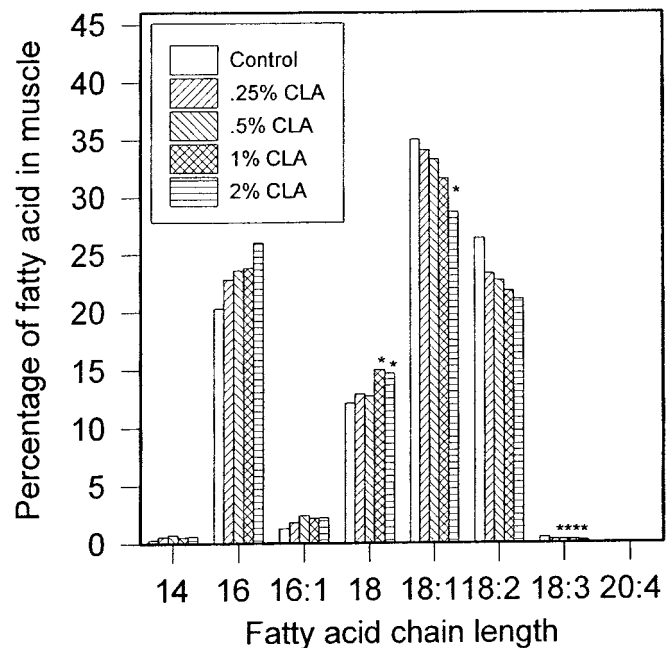
Delany et al., (1999) suggested that an elevation in serum free fatty acids could contribute to the relative insulin resistance observed in mice. However, the present study did not detect any differences in serum free

fatty acids among any of the treatment groups (data not presented). Ostrowska et al. (1999b) reported that serum free fatty acid levels and triglycerides were elevated in chronically cannulated finishing pigs fed CLA. The present study did not use chronically cannulated swine. Thus, small changes in serum free fatty acid levels that have been attributed to lipolytic activity (Ostrowska et al., 1999b) could not be measured in the present study.

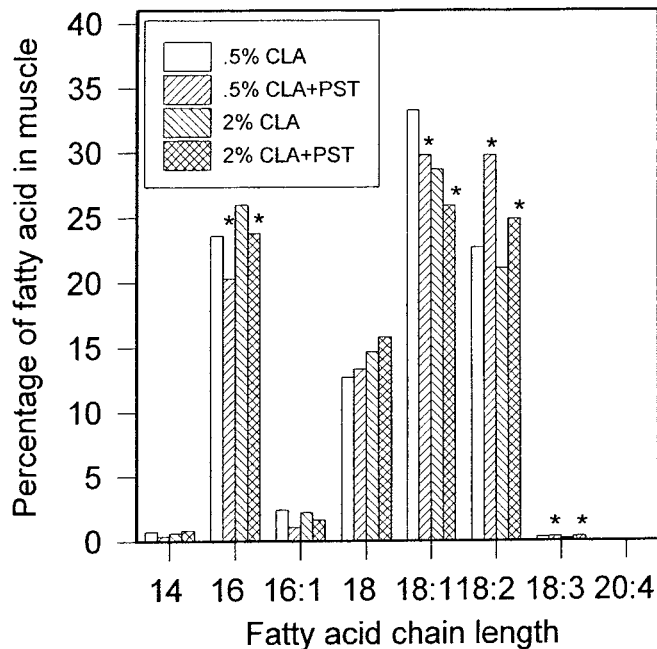
Somatotropin produced the characteristic changes in serum metabolites shown in previous studies of grower pigs (Campbell et al., 1988; Caperna et al., 1993; Evock-Clover, 1997). Treatment with pST produced insulin



**Figure 2.** Percentage of 9:11 and 10:12 conjugated linoleic acid (CLA) isomers constituting the total measurable fatty acid pool in samples of latissimus muscle from pigs fed a diet containing 0 to 2.0% CLA from 20 to 55 kg BW or from pigs injected with bicarbonate buffer and fed the basal diet (B) or injected with 100 µg porcine somatotropin/kg BW and fed the 0.5% CLA diet (0.5% + PST) or 2.0% CLA (2% + PST) ( $n = 6$ ). <sup>a,b,c,d,e</sup>Treatment means for CLA isomer 9:11 means lacking a common superscript letter differ ( $P < 0.05$ ). <sup>w,x,y,z</sup>Treatment means for CLA isomer 10:12 lacking a common superscript letter differ ( $P < 0.05$ ).



**Figure 3.** Percentage of individual fatty acids constituting the total measurable fatty acid pool in samples of latissimus muscle from pigs fed a diet containing 0 to 2.0% conjugated linoleic acid (CLA) from 20 to 55 kg BW ( $n = 6$ ;  $P < 0.05$ ). \*Different from tissue derived from animals fed 0% CLA ( $P < 0.05$ ).



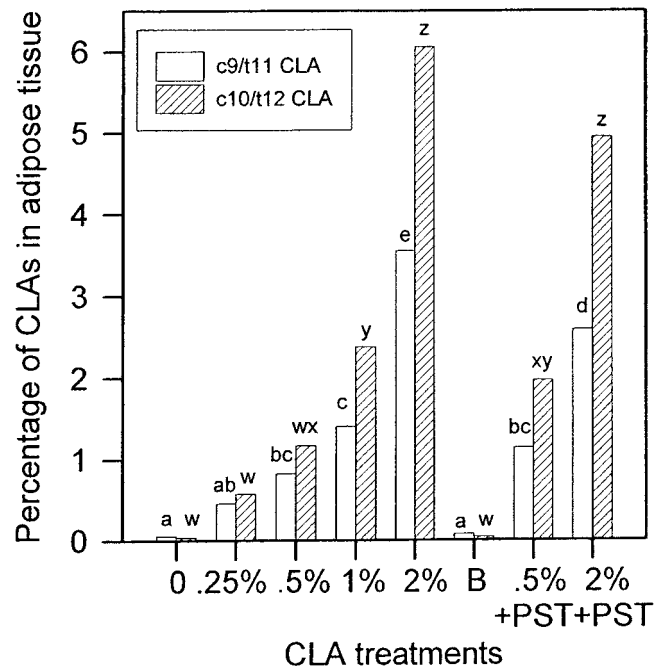
**Figure 4.** Percentage of individual fatty acids constituting the total measurable fatty acid pool in samples of latissimus muscle from pigs treated with or without porcine somatotropin (pST, 100  $\mu$ g/kg BW) and fed a diet containing either 0.5% or 2.0% conjugated linoleic acid from 20 to 55 kg BW ( $n = 6$ ;  $P < 0.05$ ). \*Different from tissue derived from animals not treated with pST ( $P < 0.05$ ).

resistance, as demonstrated by the increases in serum glucose and insulin. Wray-Cahen et al. (1987) reported that pST induced up to a 60% reduction in vivo in tissue glucose uptake in swine. A reduction in glucose utilization for lipid synthesis by adipose tissue contributes to this insulin resistance (Walton et al., 1987; Dunshea et al., 1992). Thus, it is likely that a reduction in glucose utilization and lipid synthesis within adipose tissue is responsible for the reduced carcass lipid of pST-treated pigs in the present study.

The induction of serum IGF-I by pST treatment in swine has been well described (Etherton and Smith, 1991). The change in serum IGF-I induced by pST in the present study could be segregated according to sex, with the gilts showing less of a response to pST than barrows. This corresponded with changes in serum BUN: gilts demonstrated less of a reduction in BUN than barrows with pST treatment. The lower BUN of pST-treated animals probably reflected the higher protein accretion rates, increased amino acid uptake, and concomitant decline in the amino acid pool destined for oxidation by these animals, compared to buffer-treated counterparts. Evock-Clover et al. (1992) proposed that IGF-I may more closely mediate changes in protein metabolism in swine than pST because BUN concentrations were correlated with IGF-I ( $r^2 > -0.63$ ,  $P < 0.01$ ) in that study. Thus, the difference in serum IGF-I between gilts and barrows may have contributed to the difference in BUN between the sexes.

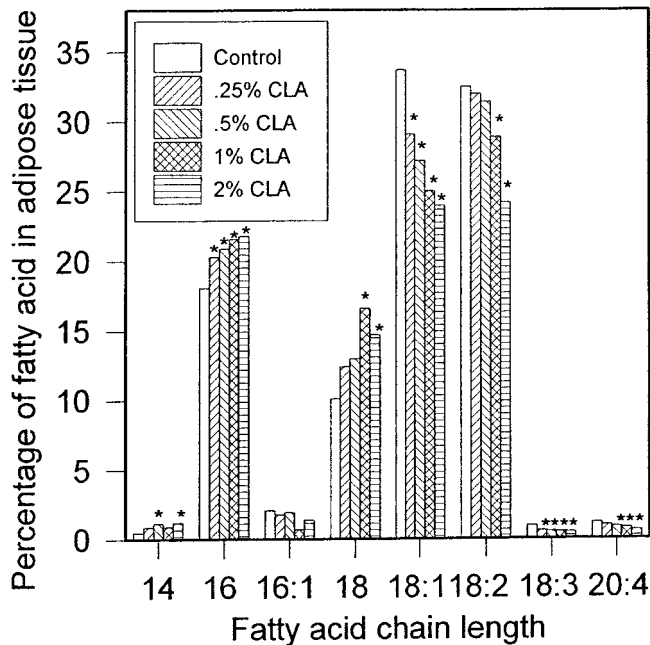
Conjugated linoleic acid treatment has been shown to reduce the quantity of carcass fat, thereby increasing the percentage of carcass lean in finishing pigs (Dugan et al., 1997; Dunshea et al., 1998; Ostrowska et al., 1999a). Ostrowska et al. (1999b) proposed that dietary CLA reduces fat accretion in finishing pigs by reducing lipogenesis from preformed fatty acids and by increasing lipolysis. Alternatively, carcass fat may be reduced by CLA-induced apoptosis and subsequent lipodystrophy within the adipose tissue (Evans et al., 2000; Tsuboyama-Kasaoka et al., 2000). This second mechanism requires further investigation, because Azain et al. (2000) reported that CLA only affects cell size (metabolism) without producing any change in cell number, arguing against apoptosis.

Again, the present study in grower pigs did not reflect findings of these previous reports. No differences in body composition were detected that could be attributed to dietary CLA supplementation. Dunshea et al. (1998) and Ostrowska et al. (1999a) reported up to a 25 to 30% reduction in s.c. backfat, whereas Dugan et al. (1997) reported a 6.5% reduction in backfat in animals weighing 100 to 105 kg. An increase in backfat thick-



**Figure 5.** Percentage of 9:11 and 10:12 conjugated linoleic acid (CLA) isomers constituting the total measurable fatty acid pool in samples of s.c. adipose tissue from pigs fed a diet containing 0 to 2.0% CLA from 20 to 55 kg BW or from pigs injected with bicarbonate buffer and fed the basal diet (B) or injected with 100  $\mu$ g porcine somatotropin/kg BW and fed the 0.5% CLA diet (0.5% + PST) or 2.0% CLA (2% + PST) ( $n = 6$ ). <sup>a,b,c,d,e</sup>Treatment means for CLA isomer 9:11 means lacking a common superscript letter differ ( $P < 0.05$ ). <sup>w,x,y,z</sup>Treatment means for CLA isomer 10:12 lacking a common superscript letter differ ( $P < 0.05$ ).





**Figure 6.** Percentage of individual fatty acids constituting the total measurable fatty acid pool in samples of dorsal s.c. adipose tissue from pigs fed a diet containing 0 to 2.0% conjugated linoleic acid (CLA) from 20 to 55 kg BW ( $n = 6$ ;  $P < 0.05$ ). \*Different from tissue derived from animals fed 0% CLA ( $P < 0.05$ ).

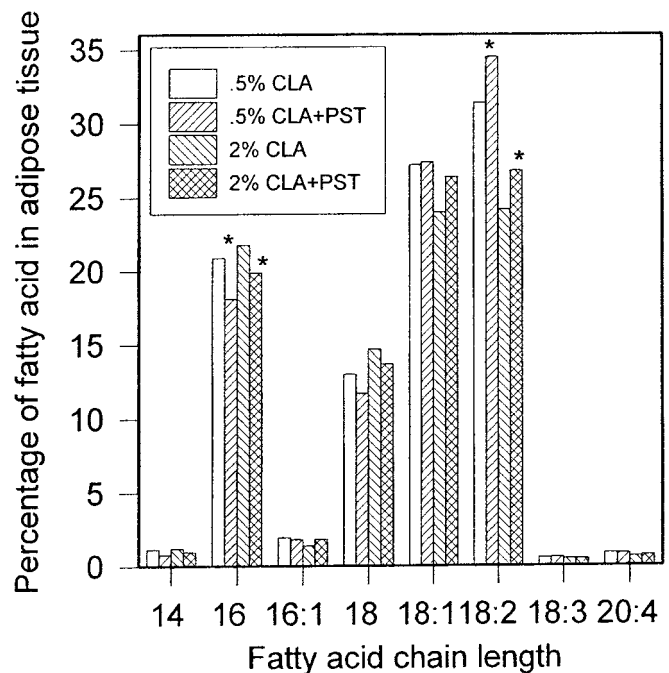
ness was observed in animals fed 0.25 or 0.5% CLA in comparison to control animals in the present study. No change in carcass lipid quantity or percentage in response to CLA was detected in the present study. Grower pigs deposit fat at a lower proportion of total gain than lean tissue (Boyd et al., 1991). Animals may need to accumulate fat at a higher rate than that in the grower pig to permit detection of an effect of dietary CLA treatment on fat accretion. Thus, this study indicated that swine should be in the finisher phase of growth to obtain the maximal beneficial effects of CLA supplementation on carcass composition.

Somatotropin produced effects on reductions in carcass lipid and water percentage in grower pigs similar to those previously reported (Evock-Clover et al., 1997). The primary effect of pST on lipid accretion seems to be inhibition of lipogenesis (Magri et al., 1990; Wolverton et al., 1992), whereas it has been suggested that CLA increases lipolysis (Park et al., 1997). The percentage of carcass protein was not affected by pST treatment, suggesting that young, growing pigs in the present study were approaching their genetic capacity for lean gain (Campbell et al., 1989).

Although no changes in adipose tissue or carcass protein accretion were detected in response to dietary CLA, tissue analysis of fatty acid profiles demonstrated that replacement of dietary corn oil with CLA affected tissue composition. Overall, the fatty acid composition of adipose tissue was more sensitive to dietary CLA substitu-

tion than the composition of skeletal muscle. This may simply be the consequence of the metabolic function of adipose tissue to accumulate fatty acids vs oxidation in skeletal muscle.

The apparent increase in the relative percentage of stearic acid and reduction in oleic acid indicated that  $\Delta^9$  stearoyl-CoA desaturase was inhibited within the skeletal muscle and adipose tissue at higher concentrations of dietary CLA (1 to 2%). Lee et al. (1998) reported that CLA reduces the stearoyl-CoA desaturase mRNA abundance in rat liver by 45%. The apparent inhibition of desaturase activity may contribute to the relative increase in the proportion of saturated:unsaturated fatty acids and thus the reported increase in firmer bellies of carcasses from pigs fed CLA (Eggert et al., 1999). This inhibition may be through a polyunsaturated fatty acid responsive element in the promoter region of stearoyl-CoA desaturase (Waters et al., 1997). Stearoyl-CoA desaturase mRNA abundance has been detected in swine tissues and reported to increase with postnatal age (Smith et al., 1999). Stearoyl-CoA desaturase enzyme activity has been detected in porcine adipose tissue (Ho et al., 1975) and bovine adipose tissue (St. John et al., 1991). Ho et al. (1975) demonstrated that the relative level of desaturase activity was dependent on copper content in swine diets. However, the diet in the present study contained copper in sufficient



**Figure 7.** Percentage of individual fatty acids constituting the total measurable fatty acid pool in samples of dorsal s.c. adipose tissue from pigs treated with or without porcine somatotropin (pST, 100  $\mu$ g/kg BW) and fed a diet containing either 0.5% or 2.0% conjugated linoleic acid from 20 to 55 kg BW and ( $n = 6$ ;  $P < 0.05$ ). \*Different from tissue derived from animals not treated with pST ( $P < 0.05$ ).

concentration (10 ppm/kg diet) to permit maximal desaturase activity.

In the present study, linolenic acid concentrations were reduced in adipose tissue and muscle by feeding CLA in replacement of corn oil. Kramer et al., (2000) have reported that feeding CLA reduces the linolenic acid concentration of pig liver and heart, in association with an increase in major metabolites of linolenic acid (20:5*n*-3; 22:5*n*-3, and 22:6*n*-3). Kramer et al. (2000) proposed that reductions in linolenic acid concentration are the result of CLA inhibition of *n*-6 fatty acid metabolism and CLA activation of *n*-3 fatty acid metabolism. In the present study, the decrease in tissue linoleic acid with feeding of CLA may have been in large part due to a reduction in available linoleic acid because of the replacement of corn oil with CLA. The present study suggests that nutritional studies with CLA may require supplemental linoleic acid to ensure an adequate essential fatty acid supply. Alternatively, a high-fat diet may be necessary to preclude the reduction in tissue linoleic acid content.

Somatotropin treatment of CLA-fed pigs reduced the percentage of palmitic and oleic acids relative to linoleic and linolenic fatty acids in skeletal muscle. Similarly, the percentage of palmitic acid was reduced whereas the proportion of linoleic acid was increased in s.c. adipose tissue of CLA-fed pigs treated with pST. These data suggest that somatotropin suppressed de novo fatty acid synthesis but did not affect the uptake of dietary fatty acids for use in triglyceride synthesis. The inability to alter fatty acid uptake is not surprising because pST has been shown not to inhibit lipoprotein lipase activity, although that study was performed in finishing pigs (Wolverton et al., 1992). Previous studies have demonstrated inhibition of de novo fatty acid synthesis by pST treatment (Lee et al., 2000; Magri et al., 1990; Wolverton et al., 1992) to support this hypothesis. Lee et al. (2000) and Wolverton et al. (1992) reported that lipogenesis was inhibited to a much greater extent than fatty acid esterification. This may also contribute to why the proportion of saturated fatty acids vs unsaturated fatty acids was reduced with pST treatment. Clark et al. (1992) reported that the percentage of total saturated fatty acids is reduced by 14%, whereas the total PUFA is unchanged in adipose tissue by pST treatment.

The principle goal of this experiment was to determine whether there was a synergistic effect between CLA and pST to reduce total lipid content of the carcass in grower pigs. This was not proven. Second, this experiment was designed to determine whether tissue lipid composition could be altered with CLA in the diet. Dietary CLA increased the saturated:unsaturated fatty acid ratio in skeletal muscle and adipose tissue. Last, this study demonstrated that pST reversed this shift in fatty acid composition. Somatotropin enhanced the percentage of polyunsaturated fatty acids in skeletal muscle and adipose tissue while reducing the percentage of saturated fatty acids in tissues of pigs fed CLA.

This shift in fatty acid composition, though not large, was significant, and may be useful for improving the nutritional qualities of meat from CLA-fed pigs.

## Implications

This experiment was intended to use dietary conjugated linoleic acid (CLA) supplementation in conjunction with treatment with porcine somatotropin to reduce the total lipid content and simultaneously improve and enrich the composition of the lipid in edible product. Conjugated linoleic acid failed to reduce total lipid content in the carcasses of grower pigs, suggesting that CLA may have its most beneficial effects on carcass lipid accumulation during the finishing phase. Somatotropin treatment enhanced the nutritional qualities of the muscle and fat from grower pigs fed CLA by reducing the proportion of saturated fatty acids and increasing the proportion of polyunsaturated fatty acids.

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